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ATGGCAAATA AAGCAGTAAA TGACTTTATA CTAGCTATGA 40

ATTACGATAA AAAGAAACTC TTGACCCATC AGGGAGAAAG 80

TATTGAAAAT CGTTTCATCA AAGAGGGTAA TCAGCTACCC 120

GATGAGTTTG TTGTTATCGA AAGAAAGAAG CGGAGCTTGT 160

CGACAAATAC AAGTGATATT TCTGTAACAG CTACCAACGA 200

CAGTCGCCTC TATCCTGGAG CACTTCTCGT AGTGGATGAG 240

ACCTTGTTAG AGAATAATCC CACTCTTCTT GCGGTCGATC 280

GTGCTCCGAT GACTTATAGT ATTGATTTGC CTGGTTTGGC 320

AAGTAGCGAT AGCTTTCTCC AAGTGGAAGA TCCCAGCAAT 360

TCAAGTGTTC GCGGAGCGGT AAACGATTTG TTGGCTAAGT 400

GGCATCAAGA TTATGGTCAG GTCAATAATG TCCCAGCTAG 440

AATGCAGTAT GAAAAAATCA CGGCTCACAG CATGGAACAA 480

CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGACAGGGA 520

ATTCTCTTGA TATTGATTTT AACTCTGTCC ATTCAGGCGA 560

AAAGCAGATT CAGATTGTTA ATTTTAAGCA GATTTATTAT 600

ACAGTCAGCG TAGACGCTGT TAAAAATCCA GGAGATGTGT 640

TTCAAGATAC TGTAACGGTA GAGGATTTAA AACAGAGAGG 680

AATTTCTGCA GAGCGTCCTT TGGTCTATAT TTCGAGTGTT 720

GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACGA 760

GTAAGAGTGA TGAAGTAGAG GCTGCTTTTG AAGCTTTGAT 800

AAAAGGAGTC AAGGTAGCTC CTCAGACAGA GTGGAAGCAG 840

ATTTTGGACA ATACAGAAGT GAAGGCGGTT ATTTTAGGGG 880

GCGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGGT 920

GGATATGGTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT 960

FIG. 1A

ACAGCAGATC ATCCAGGCTT GCCGATTTCC TATACAACTT 1000

CTTTTTTACG TGACAATGTA GTTGCGACCT TTCAAAATAG 1040

TACAGACTAT GTTGAGACTA AGGTTACAGC TTACAGAAAC 1080

GGAGATTTAC TGCTGGATCA TAGTGGTGCC TATGTTGCCC 1120

AATATTATAT TACTTGGAAT GAATTATCCT ATGATCATCA 1160

AGGTAAGGAA GTCTTGACTC CTAAGGCTTG GGACAGAAAT 1200

GGGCAGGATT TAACGGCTCA CTTTACCACT AGTATTCCTT 1240

TAAAAGGGAA TGTTCGTAAT CTCTCTGTCA AAATTAGAGA 1280

GTGTACCGGG CTTGCTTGGG AATGGTGGCG TACGGTTTAT 1320

GAAAAAACCG ATTTGCCACT AGTGCGTAAG CGGACGATTT 1360

CTATTTGGGG AACAACTCTC TATCCGCAGG TAGAAGATAA 1400

GGTAGAAAAT GAC 1413

FIG. 1B

ATGGCAAATA AAGCAGTAAA TGACTTTATA CTAGCTATGA 40

ATTACGATAN$_{50}$ AAAN$_{54}$AAACTC TTGACCCATC AGGGAGAAAG 80

TATTGAAAAT CGTTTCAN$_{98}$CA AAGAGGGTAA TCAGCTACCC 120

GN$_{122}$TGAGTTTG TTGN$_{134}$TAN$_{137}$CGA AAGAAAGAAG CGGAGCTTGT 160

CGACAAATAC AAGTGATATT N$_{181}$CTGTAN$_{187}$CAG CTACCN$_{196}$ACGA 200

CAGTCGCCTC TATCCTGGAG CACTTCTCGT AGTGGATGAG 240

ACCTTGTN$_{248}$AG AGAATAATCC CACTCTTCTT GCGGTN$_{276}$GATC 280

GTGCTCCGAT GACTTATAGT AN$_{302}$TGN$_{305}$TTTGC CTGGTTTGGC 320

AAGTAGCGAT AGCTTTCTCC AAGTGGAAGA N$_{351}$CCCAGCAAT 360

TCAAGTGTTC GCGGAGCGGN$_{380}$ AN$_{382}$ACGATTTG TTGGCTAAGT 400

GGCATCAAGA TTATGGTCAG GTCAATAATG TCCCAGCTAG 440

AAN$_{443}$GCAGTAT GAAAAAATN$_{459}$A CGGCTCACAG CATGGAACAA 480

CTCAAGGTCA AGTTTGGTTC TGACTTTGAA AAGN$_{514}$CAGGGA 520

ATTCTCTTGA TATTGATTTT AACTCTGTCC ATTCAGGN$_{558}$GA 560

AAAGCN$_{566}$GATT CAGATTGTTA ATN$_{583}$TTAAGCA GATTTATTAT 600

ACAGTCAGCG TAGACGCTGT TAAAAATCCA GGAGATGTGT 640

TTCAAGATAC TGTAACGGTA GAGGATTTAA AACAGAGAGG 680

AATTTCTGCA GAGCGTCCTT TGGTCTATAT TTCGAGN$_{717}$GTT 720

GCTTATGGGC GCCAAGTCTA TCTCAAGTTG GAAACCACGA 760

GTAN$_{764}$GAGTGN$_{770}$ TGAAGTAGAG GCTGCTTTTG AAGCTTTGAT 800

AAAAGGAGTC AAGGTAGCTC CTCAGACAGA GTGGAAGCAG 840

ATTTTGGACA ATACAGAAGT GAAGGCGGTT ATTTTAGGGG 880

GCGACCCAAG TTCGGGTGCC CGAGTTGTAA CAGGCAAGGT 920

GGATATGGTA GAGGACTTGA TTCAAGAAGG CAGTCGCTTT 960

ACAGCAGATC ATCCAGGCTT GCCGATTTCC TATACAACTT 1000

FIG. 2A

CTTTTTTACG TGACAATGTA GTTGCGACCT TTCAAAAN$_{1038}$AG 1040

TACAGACTAT GTTGAGACTA AGGTTACAGC TTACAGAAAC 1080

GGAGATTTAC TGCTGGATCA TAGTGGTGCC TATGTTGCCC 1120

AATATTATAT TACTTGGN$_{1138}$AT GAATTATCCT ATGATCATCA 1160

AGGTAAGGAA GTCTTGACTC CTAAGGCTTG GGACAGAAAT 1200

GGGCAGGATT TN$_{1212}$ACGGCTCA CTTTACCACT AGTATTCCTT 1240

TAAAAGGGAA TGTTCGTAAT CTCTCTGTCA AAATTAGAGA 1280

GTGTACCGGG CTTGCN$_{1296}$TGGG AATGGTGGCG TACGGTTTAT 1320

GAAAAAACCG ATTTGCCACT AGTGCGTAAG CGGACGATTT 1360

CTATTTGGGG AACAACTCTC TATCCN$_{1386}$CAGG TAGAN$_{1395}$GATAA 1400

GGTAGAAAAT GAC 1413

FIG. 2B

```
Met Ala Asn Lys Ala Val Asn Asp Phe Ile Leu Ala
1               5                  10
Met Asn Tyr Asp Lys Lys Lys Leu Leu Thr His Gln
            15                  20
Gly Glu Ser Ile Glu Asn Arg Phe Ile Lys Glu Gly
 25                  30                  35
Asn Gln Leu Pro Asp Glu Phe Val Val Ile Glu Arg
                40                  45
Lys Lys Arg Ser Leu Ser Thr Asn Thr Ser Asp Ile
     50                  55                  60
Ser Val Thr Ala Thr Asn Asp Ser Arg Leu Tyr Pro
                65                  70
Gly Ala Leu Leu Val Val Asp Glu Thr Leu Leu Glu
            75                  80
Asn Asn Pro Thr Leu Leu Ala Val Asp Arg Ala Pro
 85                  90                  95
Met Thr Tyr Ser Ile Asp Leu Pro Gly Leu Ala Ser
            100                 105
Ser Asp Ser Phe Leu Gln Val Glu Asp Pro Ser Asn
    110                 115                 120
Ser Ser Val Arg Gly Ala Val Asn Asp Leu Leu Ala
                125                 130
Lys Trp His Gln Asp Tyr Gly Gln Val Asn Asn Val
            135                 140
Pro Ala Arg Met Gln Tyr Glu Lys Ile Thr Ala His
145                 150                 155
Ser Met Glu Gln Leu Lys Val Lys Phe Gly Ser Asp
                160                 165
Phe Glu Lys Thr Gly Asn Ser Leu Asp Ile Asp Phe
    170                 175                 180
Asn Ser Val His Ser Gly Glu Lys Gln Ile Gln Ile
                185                 190
Val Asn Phe Lys Gln Ile Tyr Tyr Thr Val Ser Val
            195                 200
Asp Ala Val Lys Asn Pro Gly Asp Val Phe Gln Asp
205                 210                 215
Thr Val Thr Val Glu Asp Leu Lys Gln Arg Gly Ile
                220                 225
Ser Ala Glu Arg Pro Leu Val Tyr Ile Ser Ser Val
    230                 235                 240
Ala Tyr Gly Arg Gln Val Tyr Leu Lys Leu Glu Thr
                245                 250
Thr Ser Lys Ser Asp Glu Val Glu Ala Ala Phe Glu
            255                 260
Ala Leu Ile Lys Gly Val Lys Val Ala Pro Gln Thr
265                 270                 275
Glu Trp Lys Gln Ile Leu Asp Asn Thr Glu Val Lys
                280                 285
```

FIG. 3A

```
Ala Val Ile Leu Gly Gly Asp Pro Ser Ser Gly Ala
    290                 295                 300
Arg Val Val Thr Gly Lys Val Asp Met Val Glu Asp
                305                 310
Leu Ile Gln Glu Gly Ser Arg Phe Thr Ala Asp His
        315                 320
Pro Gly Leu Pro Ile Ser Tyr Thr Thr Ser Phe Leu
325                 330                 335
Arg Asp Asn Val Val Ala Thr Phe Gln Asn Ser Thr
            340                 345
Asp Tyr Val Glu Thr Lys Val Thr Ala Tyr Arg Asn
    350                 355                 360
Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val
                365                 370
Ala Gln Tyr Tyr Ile Thr Trp Asn Glu Leu Ser Tyr
        375                 380
Asp His Gln Gly Lys Glu Val Leu Thr Pro Lys Ala
385                 390                 395
Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His Phe
            400                 405
Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn
    410                 415                 420
Leu Ser Val Lys Ile Arg Glu Cys Thr Gly Leu Ala
                425                 430
Trp Glu Trp Trp Arg Thr Val Tyr Glu Lys Thr Asp
        435                 440
Leu Pro Leu Val Arg Lys Arg Thr Ile Ser Ile Trp
445                 450                 455
Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val
            460                 465
Glu Asn Asp
    470
```

FIG. 3B

```
Met Ala Asn Lys Ala Val Asn Asp Phe Ile Leu Ala
1               5                  10
Met Asn Tyr Asp Xaa Xaa Lys Leu Leu Thr His Gln
            15                  20
Gly Glu Ser Ile Glu Asn Arg Phe Xaa Lys Glu Gly
 25                  30                  35
Asn Gln Leu Pro Xaa Glu Phe Val Xaa Xaa Glu Arg
                40                  45
Lys Lys Arg Ser Leu Ser Thr Asn Thr Ser Asp Ile
     50                  55                  60
Xaa Val Xaa Ala Thr Xaa Asp Ser Arg Leu Tyr Pro
                65                  70
Gly Ala Leu Leu Val Val Asp Glu Thr Xaa Leu Glu
            75                  80
Asn Asn Pro Thr Leu Leu Ala Val Asp Arg Ala Pro
 85                  90                  95
Met Thr Tyr Ser Xaa Xaa Leu Pro Gly Leu Ala Ser
               100                 105
Ser Asp Ser Phe Leu Gln Val Glu Asp Pro Ser Asn
    110                 115                 120
Ser Ser Val Arg Gly Ala Xaa Xaa Asp Leu Leu Ala
               125                 130
Lys Trp His Gln Asp Tyr Gly Gln Val Asn Asn Val
            135                 140
Pro Ala Arg Xaa Gln Tyr Glu Lys Xaa Thr Ala His
145                 150                 155
Ser Met Glu Gln Leu Lys Val Lys Phe Gly Ser Asp
               160                 165
Phe Glu Lys Xaa Gly Asn Ser Leu Asp Ile Asp Phe
    170                 175                 180
Asn Ser Val His Ser Gly Glu Lys Xaa Ile Gln Ile
               185                 190
Val Asn Xaa Lys Gln Ile Tyr Tyr Thr Val Ser Val
            195                 200
Asp Ala Val Lys Asn Pro Gly Asp Val Phe Gln Asp
205                 210                 215
Thr Val Thr Val Glu Asp Leu Lys Gln Arg Gly Ile
               220                 225
Ser Ala Glu Arg Pro Leu Val Tyr Ile Ser Xaa Val
    230                 235                 240
Ala Tyr Xaa Arg Gln Val Tyr Leu Lys Leu Glu Thr
               245                 250
Thr Ser Xaa Ser Xaa Glu Val Glu Ala Ala Phe Glu
            255                 260
Ala Leu Ile Lys Gly Val Lys Val Ala Pro Gln Thr
265                 270                 275
Glu Trp Lys Gln Ile Leu Asp Asn Thr Xaa Val Lys
               280                 285
```

FIG. 4A

```
Ala Val Ile Leu Gly Gly Asp Pro Ser Ser Gly Ala
    290                 295                 300
Arg Val Val Thr Gly Lys Val Asp Met Val Glu Asp
                305                 310
Leu Ile Gln Glu Gly Ser Arg Phe Thr Ala Asp His
        315                 320
Pro Gly Leu Pro Ile Ser Tyr Thr Thr Ser Phe Leu
325                 330                 335
Arg Asp Asn Val Val Ala Thr Phe Gln Asn Ser Thr
            340                 345
Asp Tyr Val Glu Thr Lys Val Thr Ala Tyr Arg Asn
    350                 355                 360
Gly Asp Leu Leu Leu Asp His Ser Gly Ala Tyr Val
                365                 370
Ala Gln Tyr Tyr Ile Thr Trp Xaa Glu Leu Ser Tyr
        375                 380
Asp His Gln Gly Lys Glu Val Leu Thr Pro Lys Ala
385                 390                 395
Trp Asp Arg Asn Gly Gln Asp Leu Thr Ala His Phe
            400                 405
Thr Thr Ser Ile Pro Leu Lys Gly Asn Val Arg Asn
    410                 415                 420
Leu Ser Val Lys Ile Arg Glu Cys Thr Gly Leu Ala
                425                 430
Trp Glu Trp Trp Arg Thr Val Tyr Glu Lys Thr Asp
        435                 440
Leu Xaa Leu Val Arg Lys Arg Thr Ile Ser Ile Trp
445                 450                 455
Gly Thr Thr Leu Tyr Pro Gln Val Glu Asp Lys Val
            460                 465
Glu Asn Asp
    470
```

FIG. 4B

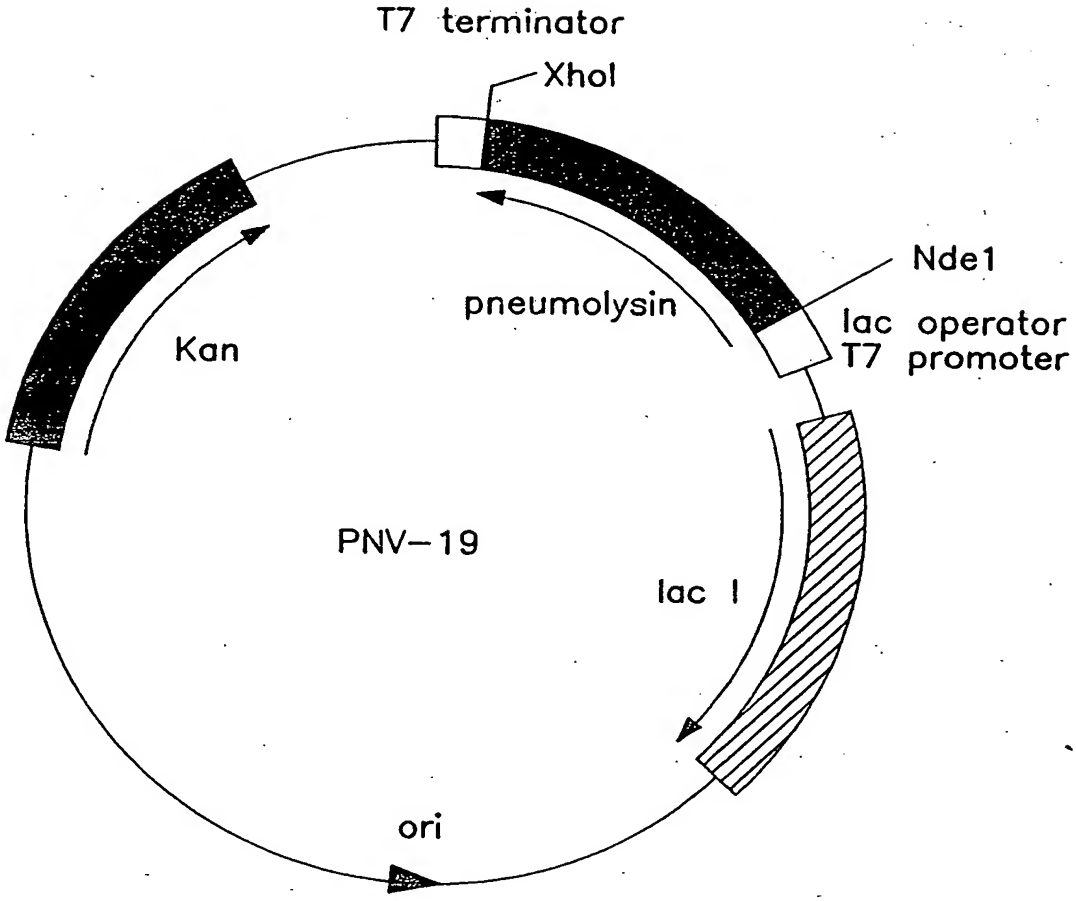
T7 terminator
XhoI
Nde1
pneumolysin
lac operator
T7 promoter
Kan
PNV–19
lac I
ori

FIG. 5

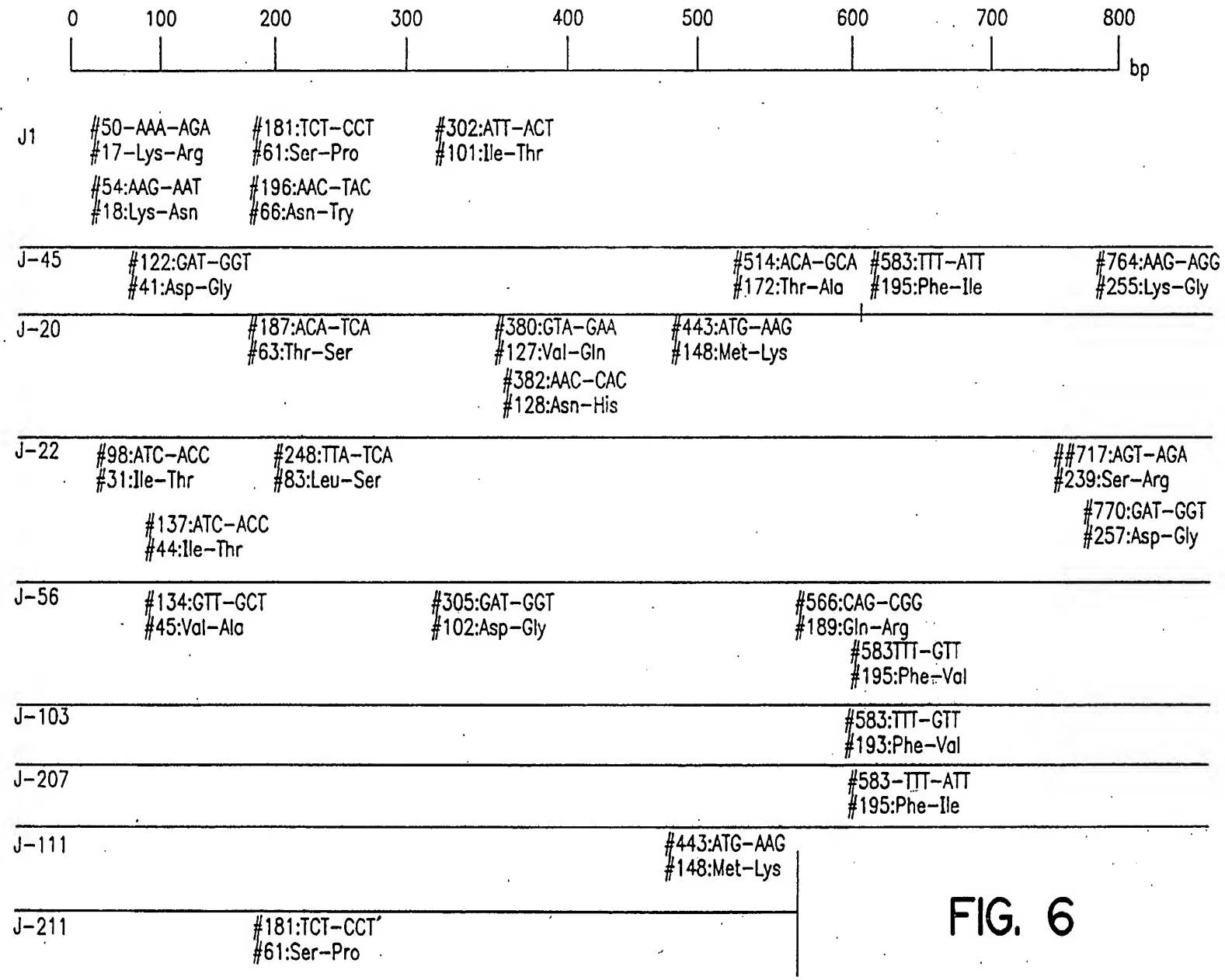
0
100
200
300
400
500
600
700
800
bp
J1
#50-AAA-AGA
#17-Lys-Arg
#181:TCT-CCT
#61:Ser-Pro
#302:ATT-ACT
#101:Ile-Thr
#54:AAG-AAT
#18:Lys-Asn
#196:AAC-TAC
#66:Asn-Try
J-45
#122:GAT-GGT
#41:Asp-Gly
#514:ACA-GCA
#172:Thr-Ala
#583:TTT-ATT
#195:Phe-Ile
#764:AAG-AGG
#255:Lys-Gly
J-20
#187:ACA-TCA
#63:Thr-Ser
#380:GTA-GAA
#127:Val-Gln
#443:ATG-AAG
#148:Met-Lys
#382:AAC-CAC
#128:Asn-His
J-22
#98:ATC-ACC
#31:Ile-Thr
#248:TTA-TCA
#83:Leu-Ser
##717:AGT-AGA
#239:Ser-Arg
#137:ATC-ACC
#44:Ile-Thr
#770:GAT-GGT
#257:Asp-Gly
J-56
#134:GTT-GCT
#45:Val-Ala
#305:GAT-GGT
#102:Asp-Gly
#566:CAG-CGG
#189:Gln-Arg
#583TTT-GTT
#195:Phe-Val
J-103
#583:TTT-GTT
#193:Phe-Val
J-207
#583-TTT-ATT
#195:Phe-Ile
J-111
#443:ATG-AAG
#148:Met-Lys
J-211
#181:TCT-CCT
#61:Ser-Pro

FIG. 6

Expression of pneumolysin

Lane 1: MW standards

Lane 2: Before IPTG induction

Lane 3: 1 hr after induction

Lane 4: 3 hrs after induction

Lane 5: 5 hrs after induction

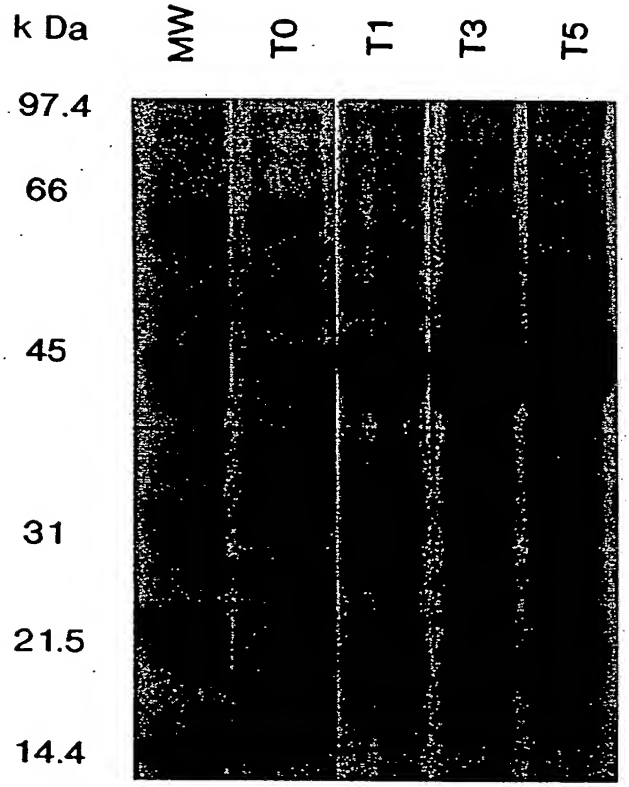

FIG. 7

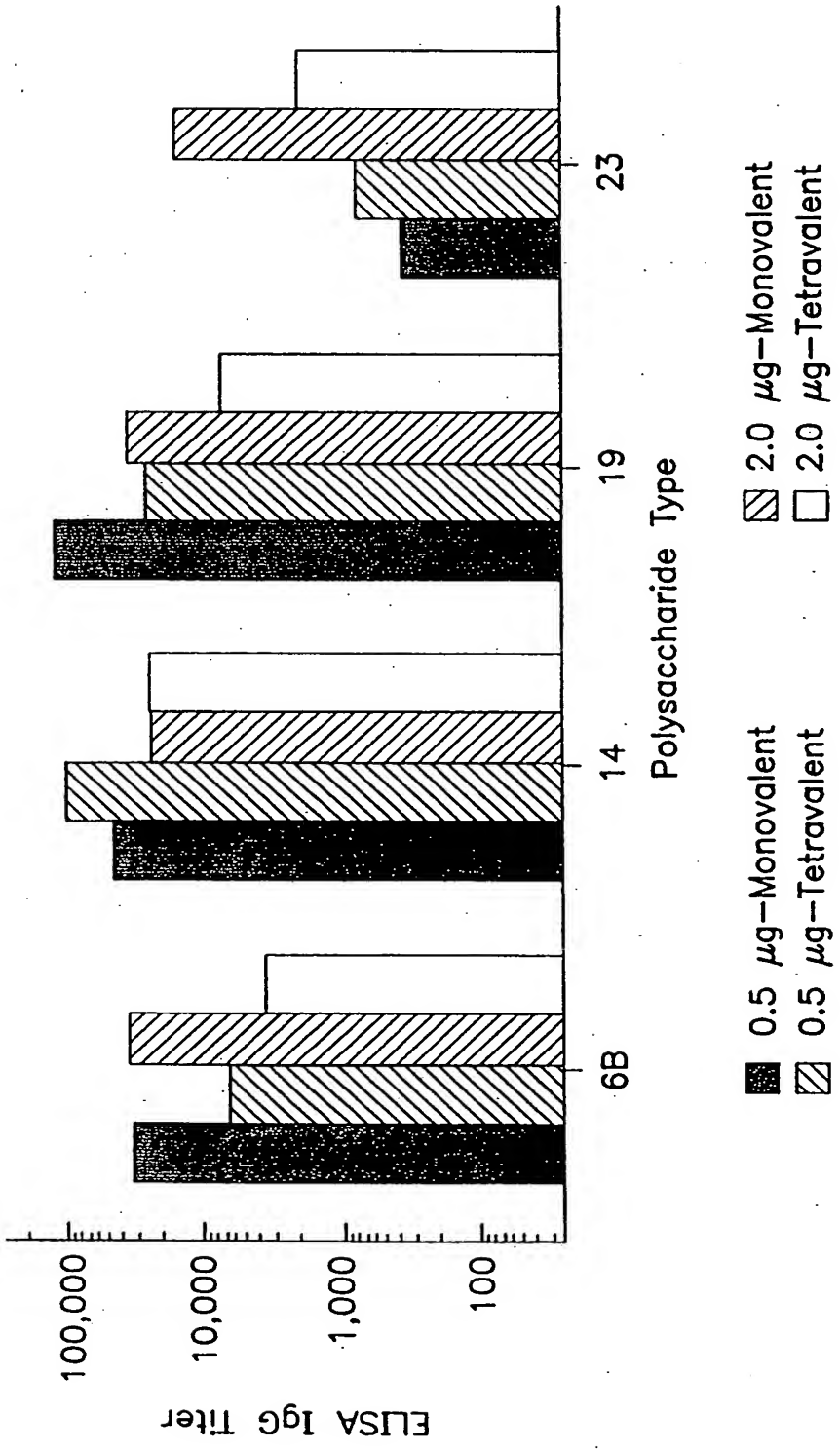
ELISA IgG Titer
100,000
10,000
1,000
100
6B
14
19
23
Polysaccharide Type
0.5 μg–Monovalent
0.5 μg–Tetravalent
2.0 μg–Monovalent
2.0 μg–Tetravalent

FIG. 8

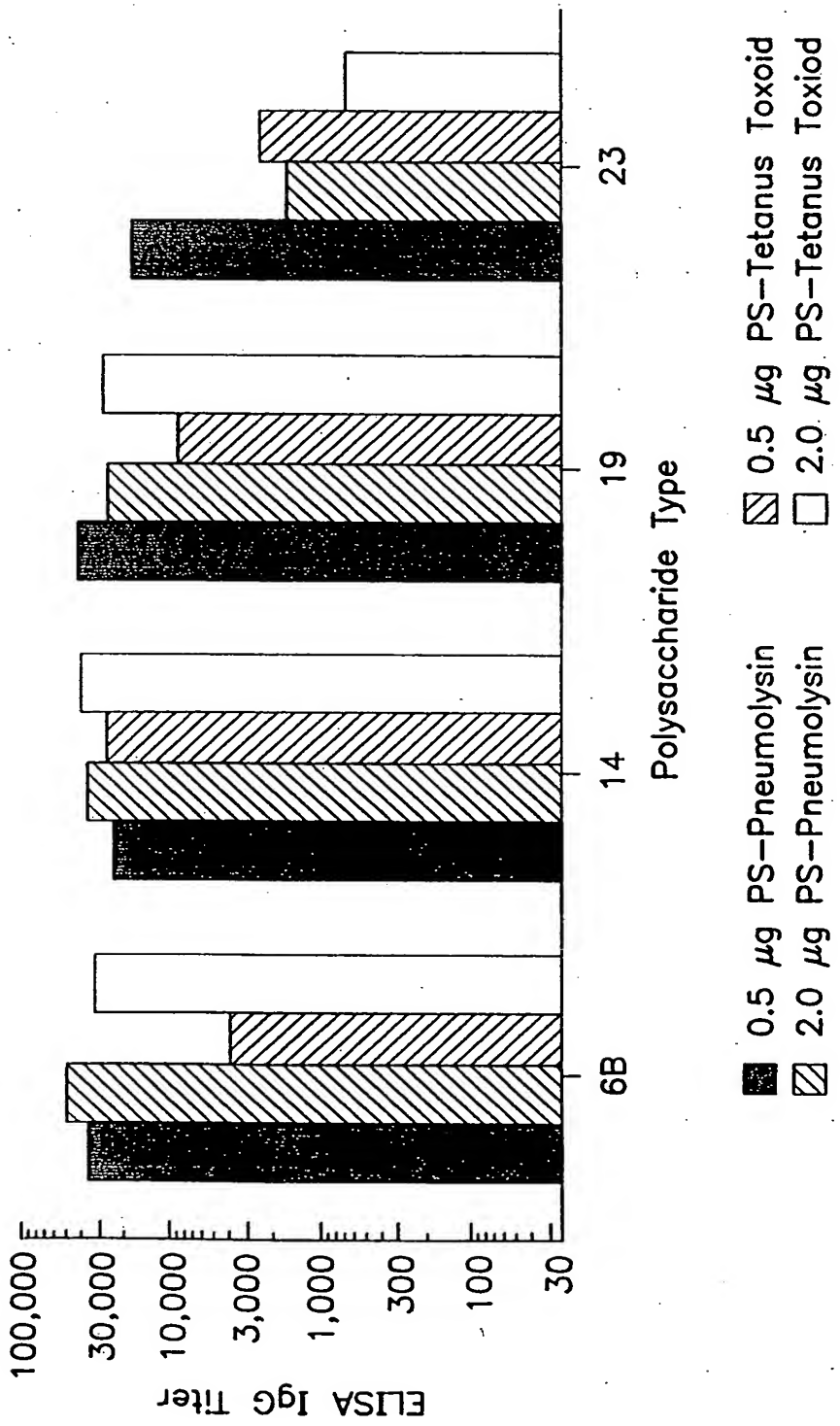
ELISA IgG Titer
100,000
30,000
10,000
3,000
1,000
300
100
30
6B
14
19
23
Polysaccharide Type
0.5 μg PS–Pneumolysin
2.0 μg PS–Pneumolysin
0.5 μg PS–Tetanus Toxoid
2.0 μg PS–Tetanus Toxiod

FIG. 9

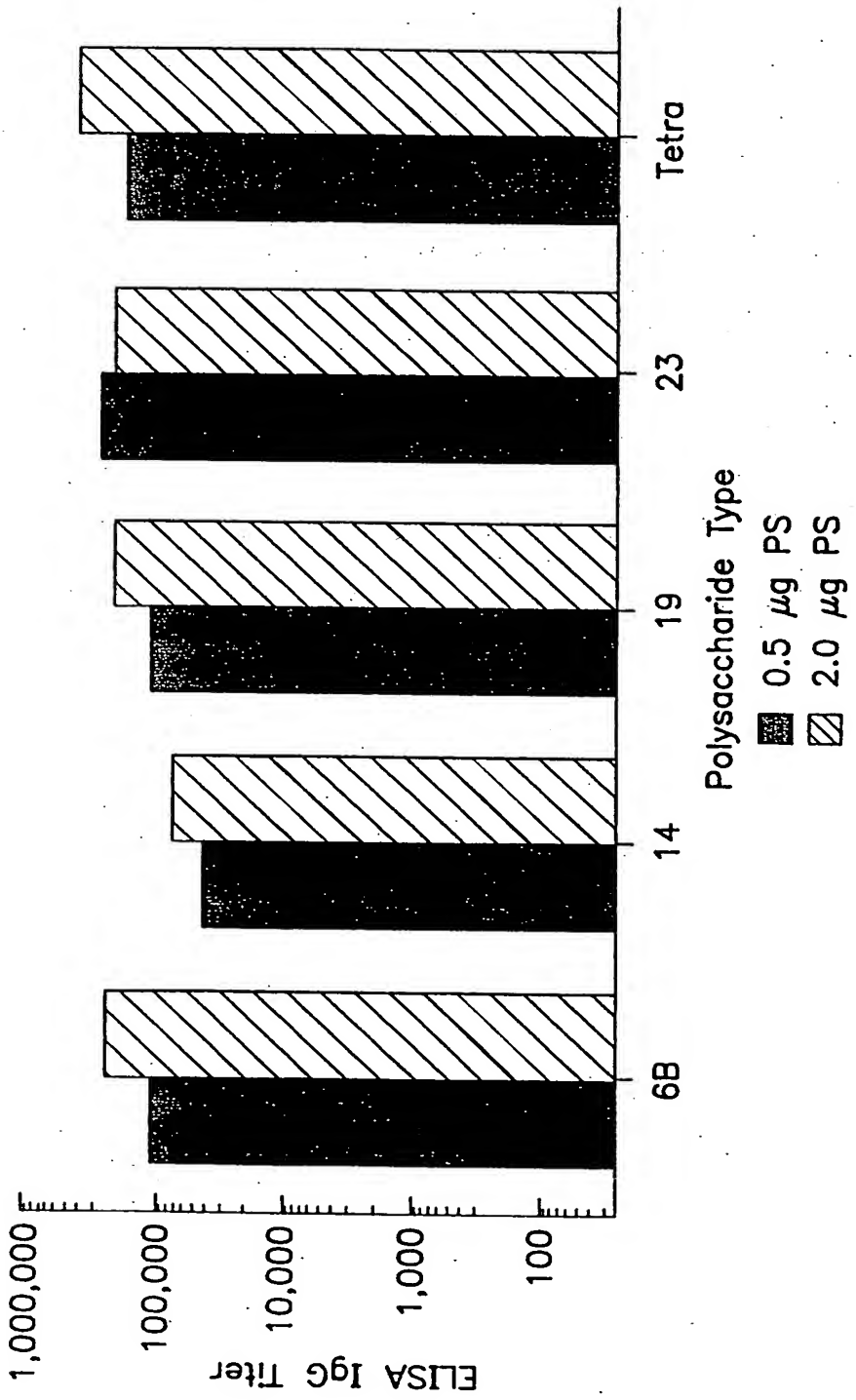
ELISA IgG Titer
1,000,000
100,000
10,000
1,000
100
6B
14
19
23
Tetra
Polysaccharide Type
0.5 μg PS
2.0 μg PS

FIG. 10

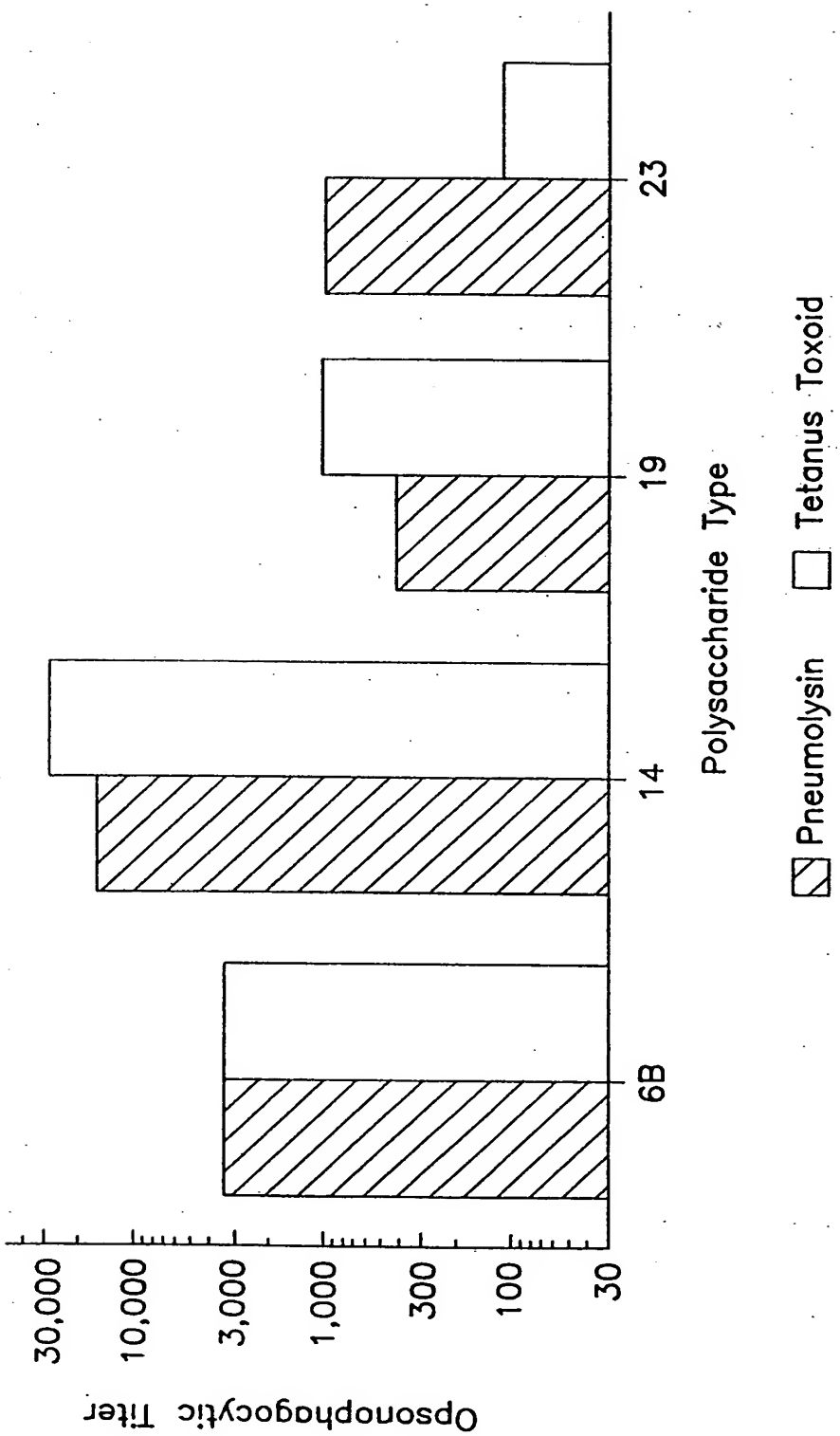
Opsonophagocytic Titer
30,000
10,000
3,000
1,000
300
100
30
6B
14
19
23
Polysaccharide Type
Pneumolysin
Tetanus Toxoid

FIG. 11

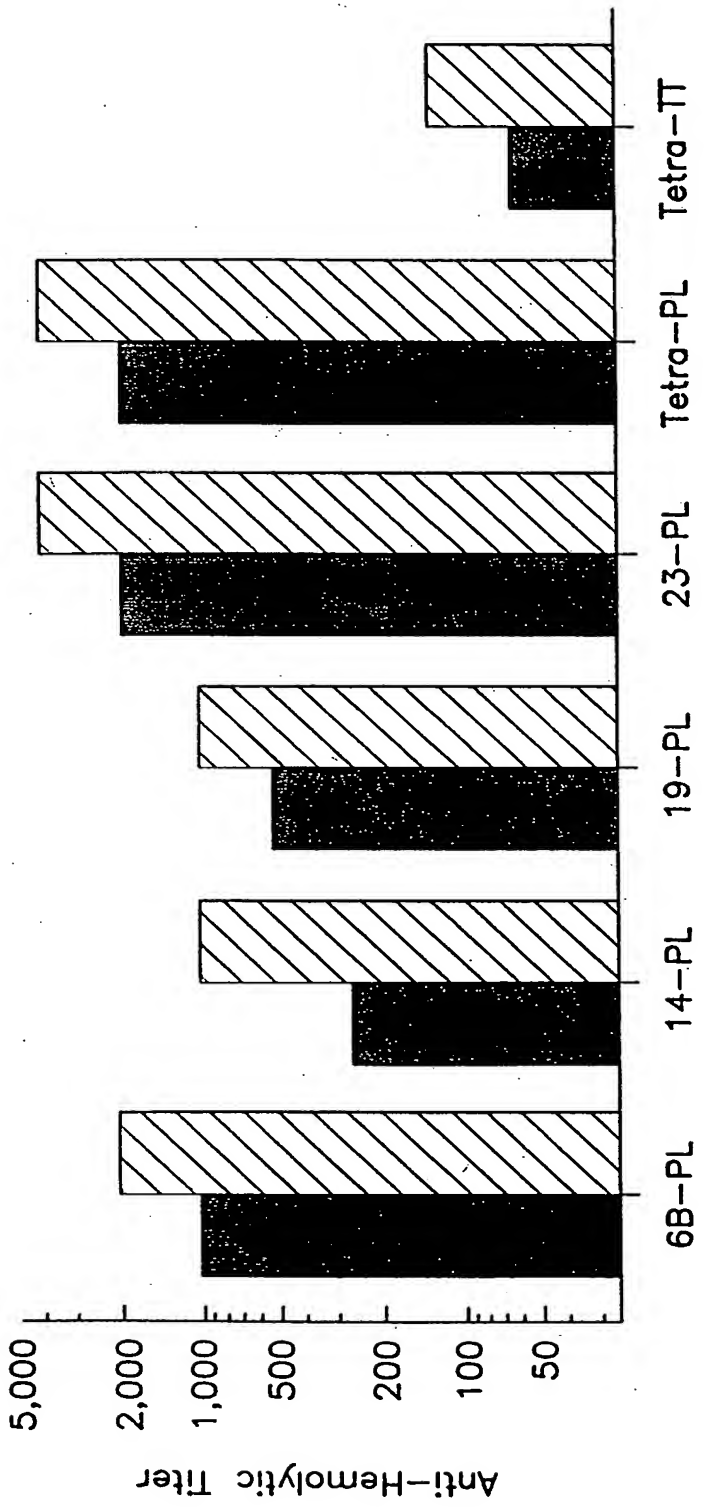
Anti-Hemolytic Titer
5,000
2,000
1,000
500
200
100
50
6B-PL
14-PL
19-PL
23-PL
Tetra-PL
Tetra-TT
Polysaccharide Type
0.5 μg PS
2.0 μg PS

FIG. 12

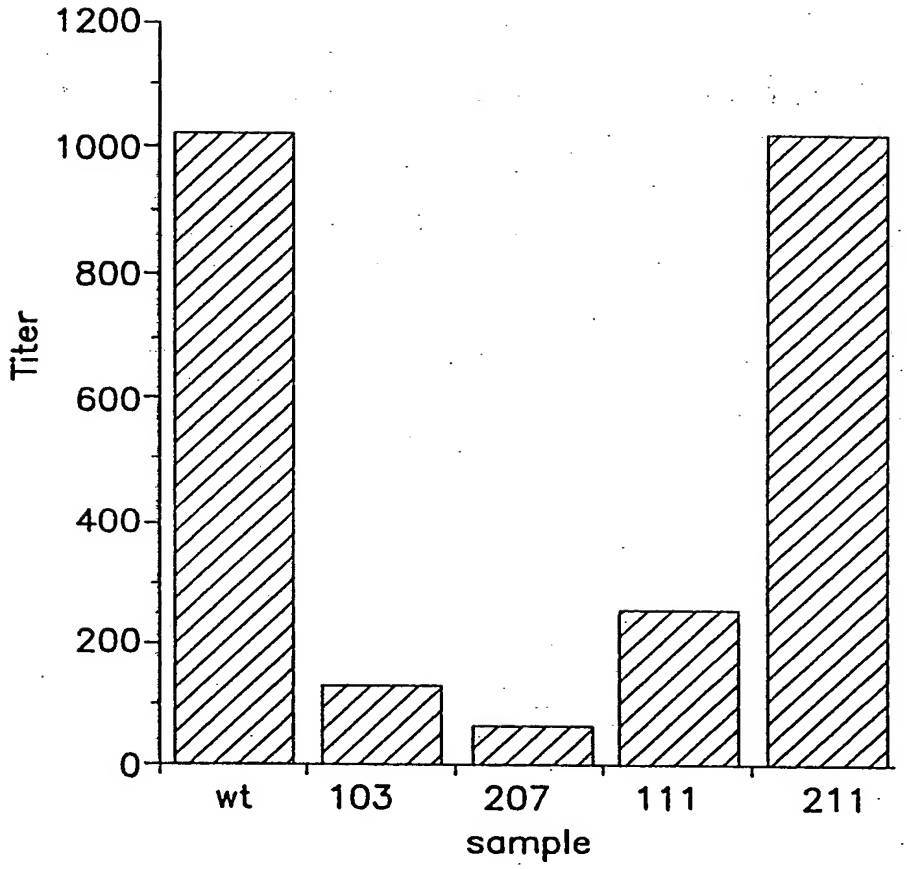

FIG. 13

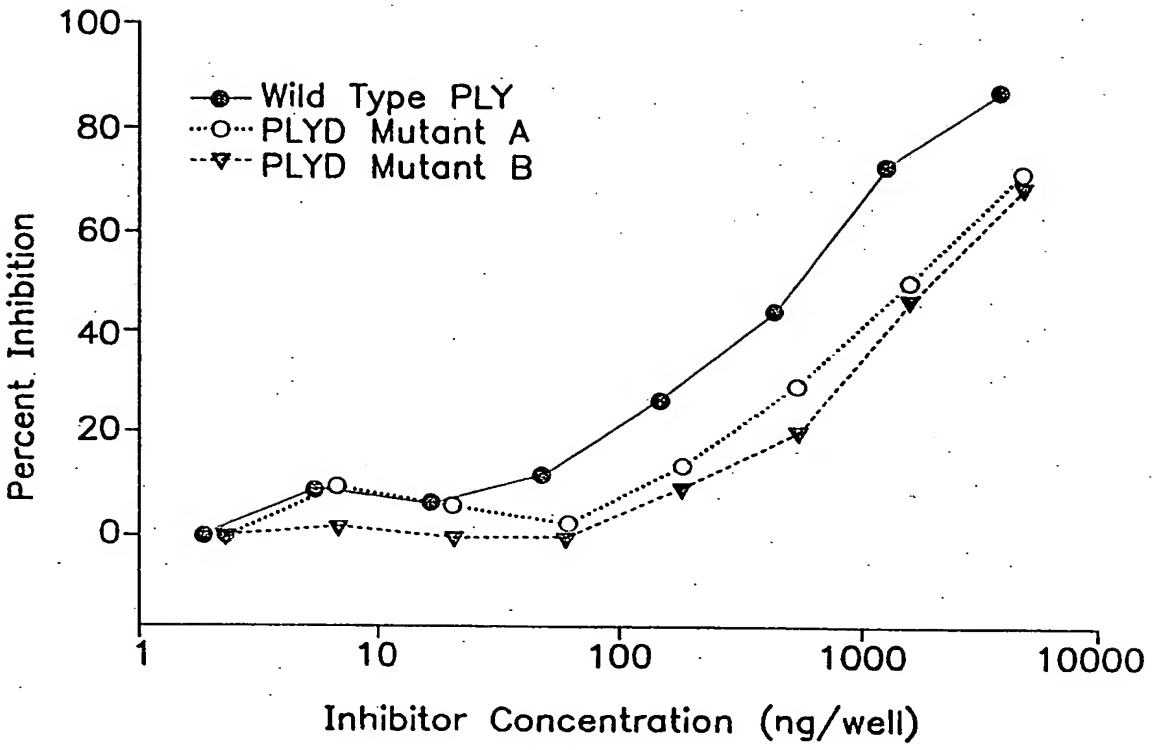
100
80
60
40
20
0
Percent Inhibition
Wild Type PLY
PLYD Mutant A
PLYD Mutant B
1
10
100
1000
10000
Inhibitor Concentration (ng/well)

FIG. 14

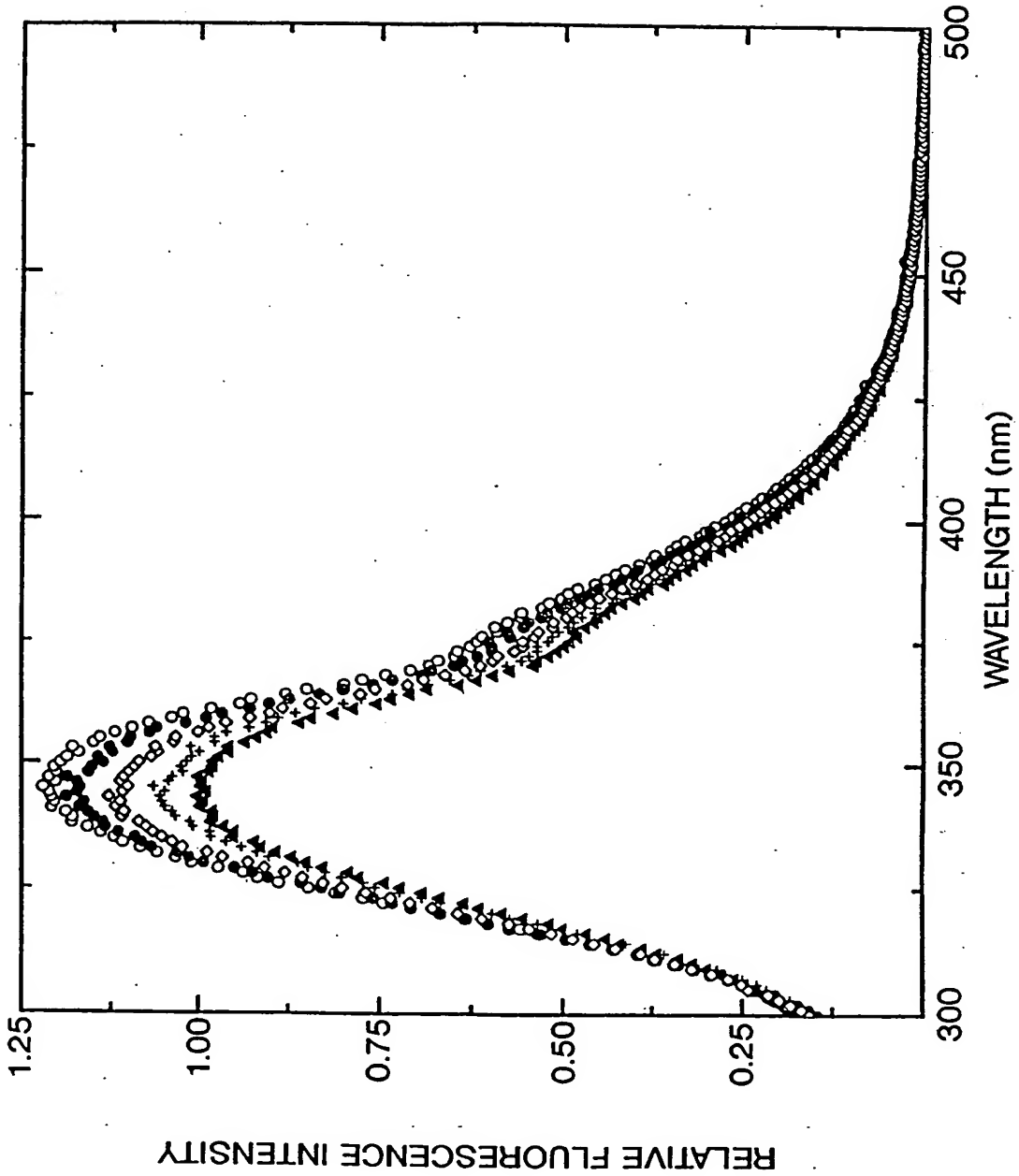
RELATIVE FLUORESCENCE INTENSITY
1.25
1.00
0.75
0.50
0.25
300
350
400
450
500
WAVELENGTH (nm)

FIG. 15

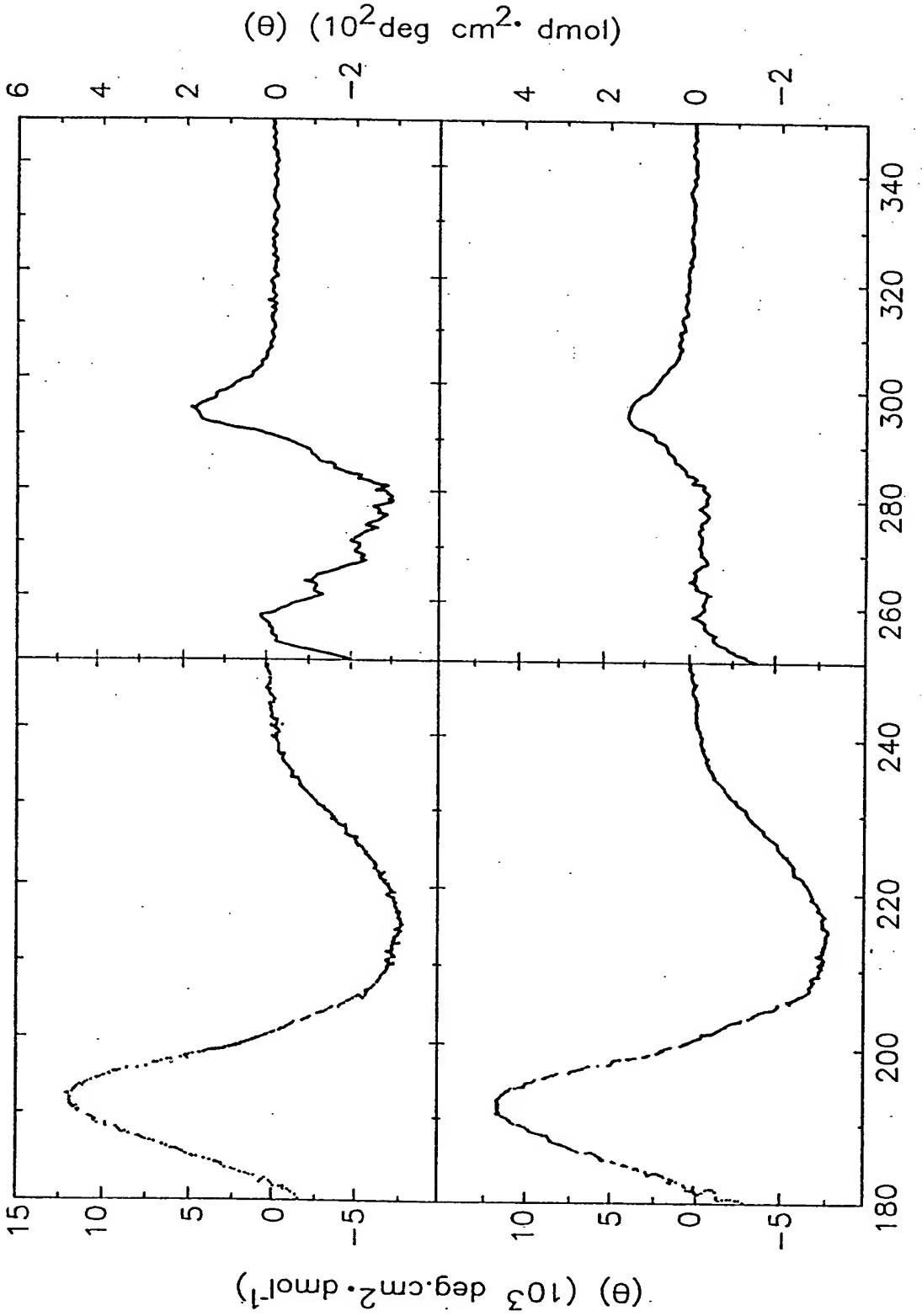

FIG. 16

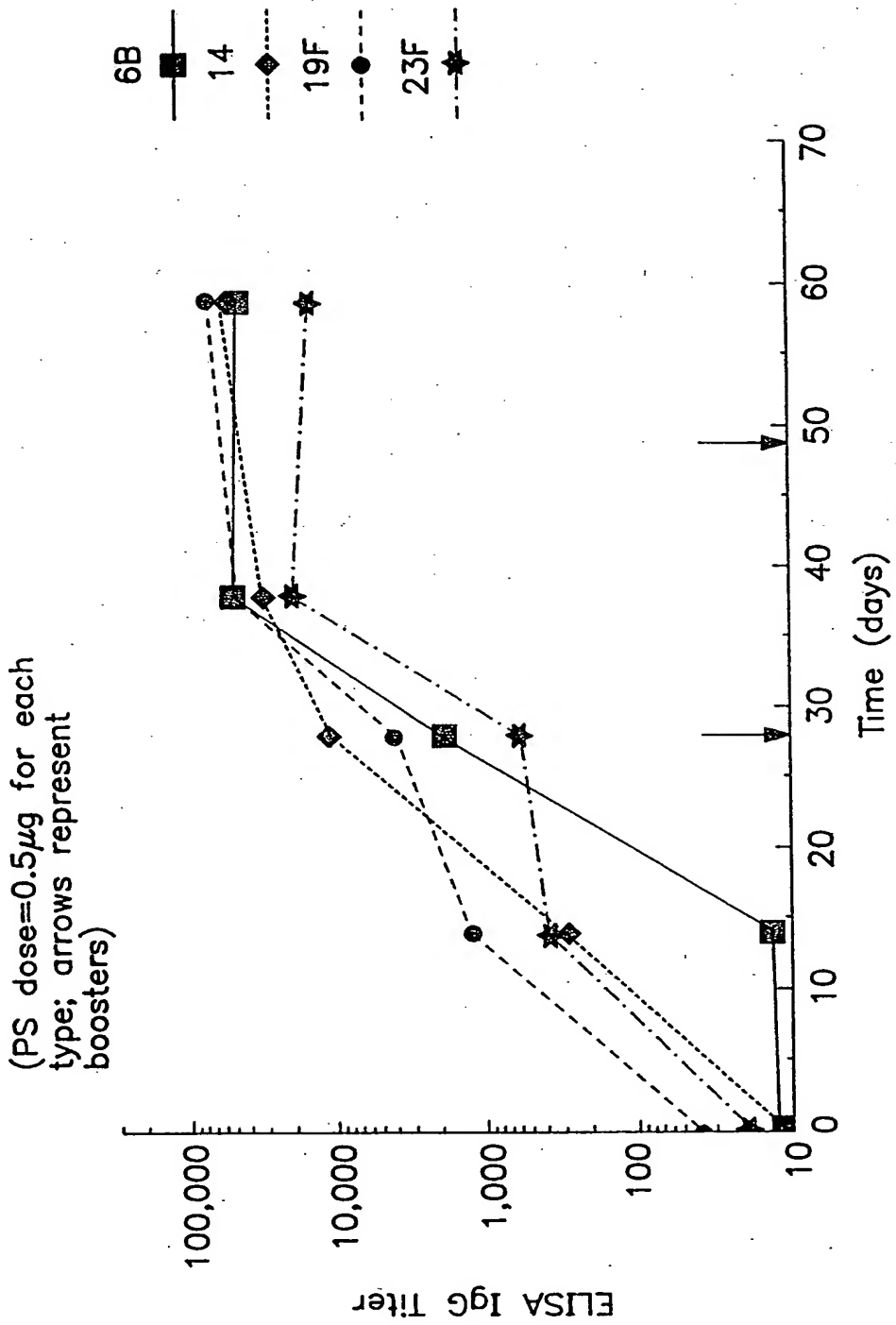
6B
14
19F
23F
(PS dose=0.5μg for each type; arrows represent boosters)
ELISA IgG Titer
100,000
10,000
1,000
100
10
0
10
20
30
40
50
60
70
Time (days)

FIG. 17A

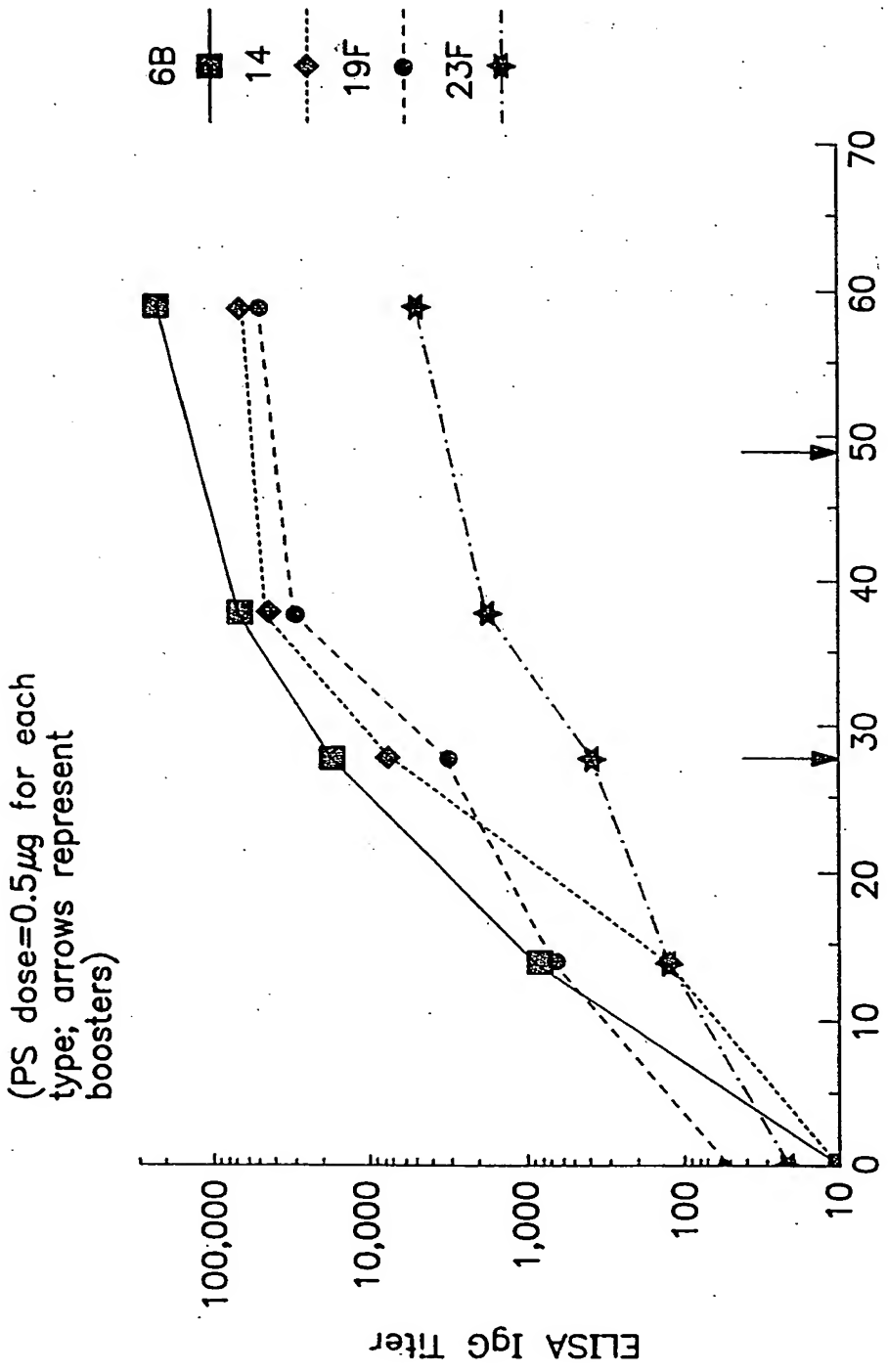
(PS dose=0.5μg for each type; arrows represent boosters)
6B
14
19F
23F
ELISA IgG Titer
100,000
10,000
1,000
100
10
0
10
20
30
40
50
60
70
Time (days)

FIG. 17B

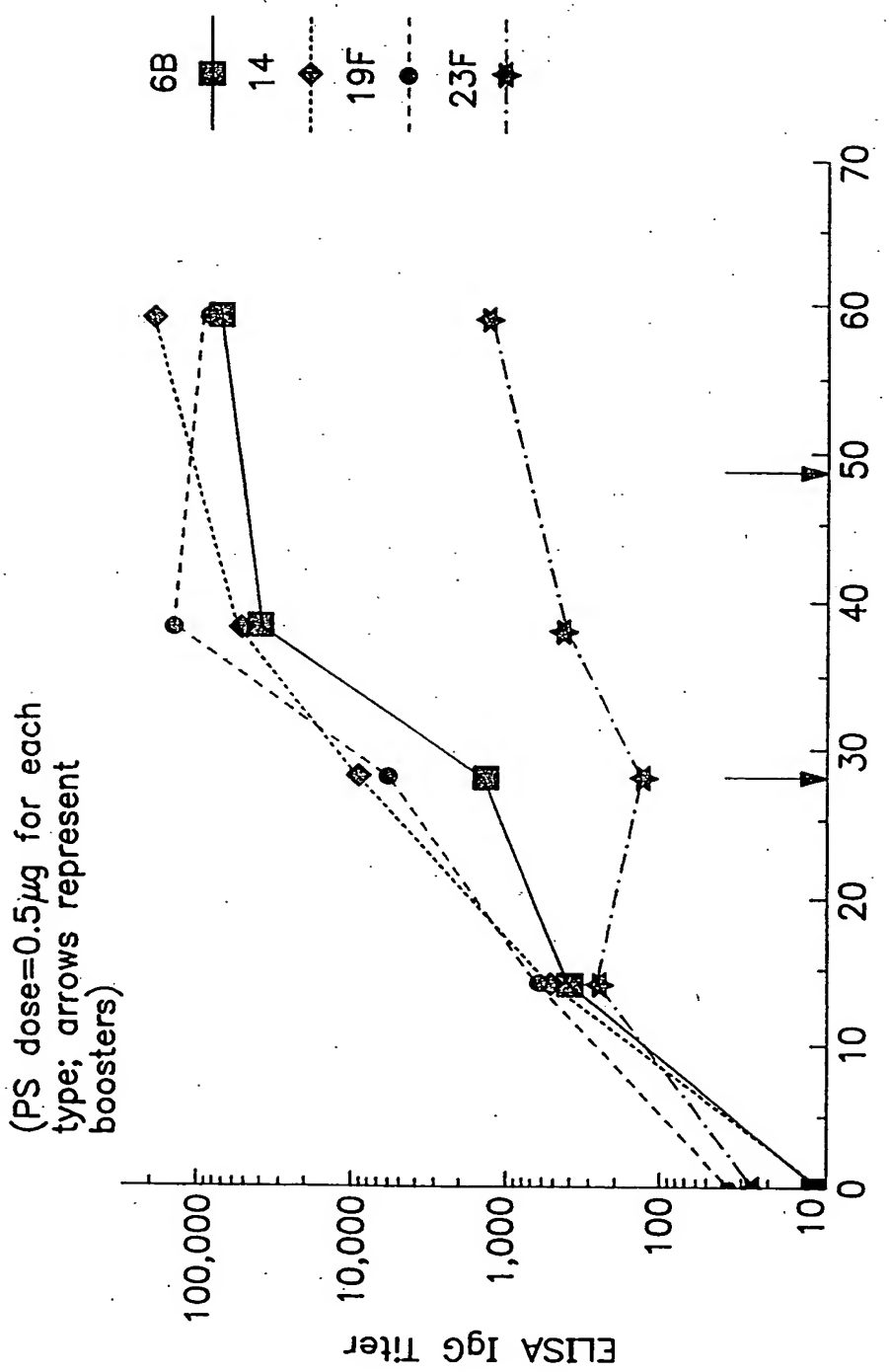
6B
14
19F
23F
(PS dose=0.5μg for each type; arrows represent boosters)
ELISA IgG Titer
100,000
10,000
1,000
100
10
0
10
20
30
40
50
60
70
Time (days)

FIG. 17C